

Evaluation of the libido of Pantaneiro stallions

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ABSTRACT - The objective with this study was to evaluate the libido and sexual behavior of Pantaneiro stallions in Brazil in two periods, the rainy and dry seasons. Their sexual behavior was evaluated during mating with mares in estrus, and the libido was scored for statistical analysis. The reaction time and time to mount and ejaculate were 78.1±64.6 s and 289.0 s, respectively. The most relevant events of sexual behavior observed in the Pantaneiro stallions were mounting without erection, mounting without ejaculation, smelling, and vocalizing. In general, the season did not affect the libido of stallions. Even with the high temperatures and humidity, which could cause thermal stress, the stallions showed good libido score during most of evaluations, with some individual differences. Pantaneiro stallions are able to breed mares during the entire year and could facilitate the breeding management to raise the number of Pantaneiro horses of the herds and optimize the use of the stallions during the breeding season, with more females per male.

Keywords: horse, reproduction, sexual behavior



Introduction

In Brazil, there is a great diversity of domestic horse breeds that were developed from others brought during colonization. Some horses brought to Brazil gradually adapted to the local environment by natural selection. Those animals left decedents and were able to spread their genes through generations, as is the case of the Pantaneiro. However, there are few studies about their reproductive capability, such as sexual behavior (Melo et al., 1998; Sereno, 2002).

The Pantaneiro horse had 26,109 registered animals until the beginning of 2019, of which 1,238 stallions were in the final record according to the Associação Brasileira de Criadores de Cavalos Pantaneiros (ABCCP, 2019).

The Pantanal is a region that covers Paraguay, Bolivia, and Brazil, with periods of heavy rains and flood. The pastures there are abundant and the temperatures are high during most of the year (Fernandes et al., 2010).

The Pantaneiro horse is very useful for farm work and for transportation, able to walk though flooded areas that are inhospitable to many animals. This breed has been at risk of extinction for years, mainly due to overly frequent crossbreeding without worrying about the purity of the breed (McManus et al., 2013). Therefore, some studies have been performed regarding genetic conservation by the ABCCP.

Therefore, some actions have been taken to understand the physiology and reproduction of this breed to improve reproductive rates and conserve the genetic material.

The sexual behavior of stallions involves events such as penis exposure, attempts to mount, smelling, biting, kicking, and vocalizing (Henry et al., 2013). These sexual events are considered to analyze the libido of the stallion, particularly the time taken for the stallion to react to a mare in estrus and the time to mount and ejaculate. The resulting libido score can be used to predict the reproductive efficiency of stallions to select them in the breeding season (McKinnon et al., 2011).

It is of great importance to study the reproductive behavior of horses from tropical regions to try to understand how they can adapt to potentially adverse environmental conditions.

The objective of this study was to observe the influence of the environment on the reproductive behavior of the Pantaneiro stallions from a tropical region of Brazil, to understand and facilitate the use of assisted reproduction to increase the population of this breed.

Material and Methods

This study was approved by the institutional committee on ethical use of animals (CEUA) in the state of Rio de Janeiro, in accordance to the standards of the Brazilian Society of Laboratory Animal Science, associated with the Brazilian College of Animal Experimentation (SBCAL/COBEA), with protocol number 245.

The study was conducted in the municipality of Poconé, in the Pantanal region, Brazil (Latitude 16°15' S, Longitude 56°37', and 80 m a.s.l.). This region has mean annual temperature of 25 °C, and from September to December the maximum temperatures can reach over 40 °C. The rainfall is variable, but abundant throughout the year, with periods of widespread flooding during the wet season.

We evaluated seven Pantaneiro stallions that were clinically healthy and had normal reproductive characteristics, based on previous evaluation. They were aged between 4 to 12 years and with reproductive experience in natural breeding. Ten evaluations were carried out for each stallion, totaling 70 observations.

The animals were kept in pastures throughout the year. Water and commercial mineral salt were available *ad libitum*. In this location, the photoperiod difference between the longest and shortest day of the year is 1 h and 54 min. The breeding soundness and other evaluations were carried out in April (end of the rainy season) and October (end of the dry season). The sexual behavior and libido of the stallions were measured by the same veterinarian.

The Pantaneiro stallions were individually guided by a person (handler) and presented to three mares in estrus (at the same time) in a corral. The stallions were allowed to perform their natural sexual behavior on approaching the mares. All were observed and the behaviors were recorded for future libido analysis.

The following behaviors were observed during the male courting of the estrous mares: disinterest in the female; interest, showing events such as smelling, biting, kicking, flehmen, vocalizing, mounting attempts, and quitting those attempts; mounting without erection (MWE), and mounting without ejaculation (MWEj).

The following actions were timed in seconds for further statistical analysis: reaction time (RT) = time from the moment when the stallions were presented to the estrous mares until penis exposure; and mounting with ejaculation time (MEjT) = time of mounting, penis introduction, ejaculation, and dismounting.

Then, the libido was evaluated by classifications: 0 – disinterest in the estrous mare; 1 – interest in the mare but quitting mounting attempts; 2 – interest in the mare and one mounting attempt without ejaculation; 3 – interest in the mare and ejaculation after several mounting attempts; 4 – high interest

in the mare and ejaculation after only a few mounting attempts; 5 – high interest in the mare and ejaculation after one mounting.

The behavior data were tabulated, and descriptive statistics were performed with the UNIVARIATE procedure and FREQ procedure in the SAS software (Statistical Analysis System, version 9.4). Analysis of the sexual behavior events and libido were performed with the Kruskal-Wallis test in the SAS to evaluate the effect of seasons (rainy season and dry season) on these traits. Spearman correlations were calculated by the CORR SPEARMAN procedure of SAS.

Results

The mean RT of the Pantaneiro stallions was 78.1 ± 64.6 s, with minimum of 8 s and maximum of 289.0 s from the moment they were presented to mares until ejaculation and dismounting. The mean time to mount, ejaculate, and dismount was 199.4 ± 166.5 s.

The median of the number of occurrences of other events, such as MWE, MWEj, flehmen, smelling, biting, kicking, and vocalizing ranged from 0 ± 0.44 to 1 ± 1.39 times performed in 70 observations of sexual behavior during semen collection using estrous mares (Table 1).

The most common sexual behavior events observed were smelling and vocalizing, with 87.1 and 85.75% frequency, respectively (Tables 2 and 3). The least common events were MWEj, biting, and kicking, with 50, 54, and 53% for zero occurrence during the semen collections, respectively.

Regarding libido, there was no difference ($P < 0.05$) of most stallions between the two seasons (rainy and dry). Only two stallions showed difference in libido between the two seasons (Figure 1).

The pairwise correlations between the sexual behavior variables were in general high (Table 4). Most of correlations between libido and the other behaviors were high and negative. Only a few correlations with libido were close to zero, such as biting and kicking, RT, MWE, MWEj, as was also the case of vocalizing with RT and MEjT.

Discussion

The mean RT of Pantaneiro stallions observed in this study (78.1 ± 64.6 s) was longer than that reported by Rua et al. (2015) for Brazilian Mangalarga Marchador stallions (11.4 ± 4.4 seconds). This difference can be attributed to the fact that Pantaneiro stallions are less often subjected to managed reproduction than Mangalarga Marchador stallions, which are used for reproduction during the entire year, with high semen collection frequency, making these animals more conditioned to breeding (Sieme et al., 2004) compared with Pantaneiro stallions. This fact could also explain the longer time of courting and tasting whether mares are in estrus by Pantaneiro stallions. Compared with other studies (Noue et al., 2001; Rua et al., 2015), we observed that the Pantaneiro stallions also took longer to mount, ejaculate, and

Table 1 - Median, variance, minimum, and maximum of the sexual behavior events of Pantaneiro stallions

| Variable | N | Median \pm S ² | Minimum | Maximum |
|------------|----|-----------------------------|---------|---------|
| MWE | 70 | 1 ± 0.94 | 0 | 4 |
| MWEj | 70 | 0 ± 0.44 | 0 | 3 |
| Flehmen | 70 | 1 ± 1.07 | 0 | 4 |
| Smelling | 70 | 1 ± 1.39 | 0 | 6 |
| Biting | 70 | 0 ± 0.39 | 0 | 2 |
| Kicking | 70 | 0 ± 0.71 | 0 | 4 |
| Vocalizing | 70 | 1 ± 1.26 | 0 | 5 |

N - Number of observations; S² - variance; MWE - mounting without erection; MWEj - mounting without ejaculation.

Table 2 - Frequency of the sexual behaviors of Pantaneiro stallions

| Variable (frequency) | N | % |
|----------------------|----|------|
| MWE | | |
| 0 | 28 | 40 |
| 1 | 22 | 31 |
| 2 | 16 | 22.9 |
| 3 | 3 | 4.3 |
| 4 | 1 | 1.4 |
| MWEj | | |
| 0 | 50 | 71 |
| 1 | 17 | 24.3 |
| 2 | 1 | 1.4 |
| 3 | 2 | 2.9 |
| Flehmen | | |
| 0 | 22 | 31.4 |
| 1 | 22 | 31.4 |
| 2 | 20 | 28.6 |
| 3 | 4 | 5.7 |
| 4 | 2 | 2.9 |
| Smelling | | |
| 0 | 9 | 12.9 |
| 1 | 28 | 40 |
| 2 | 18 | 25.7 |
| 3 | 12 | 17.1 |
| 4 | 1 | 1.4 |
| 5 | 1 | 1.4 |
| 6 | 1 | 1.4 |

N - number of observations; % - percentage of observations; MWE - mounting without erection; MWEj - mounting without ejaculation.

Table 3 - Frequency of the sexual behaviors of Pantaneiro stallions

| Variable (frequency) | N | % |
|----------------------|----|------|
| Biting | | |
| 0 | 54 | 77.1 |
| 1 | 10 | 14.3 |
| 2 | 6 | 8.6 |
| Kicking | | |
| 0 | 53 | 75.7 |
| 1 | 10 | 14.3 |
| 2 | 4 | 5.7 |
| 3 | 2 | 2.9 |
| 4 | 1 | 1.4 |
| Vocalizing | | |
| 0 | 10 | 14.3 |
| 1 | 30 | 42.9 |
| 2 | 16 | 22.9 |
| 3 | 10 | 14.3 |
| 4 | 3 | 4.3 |
| 5 | 1 | 1.4 |

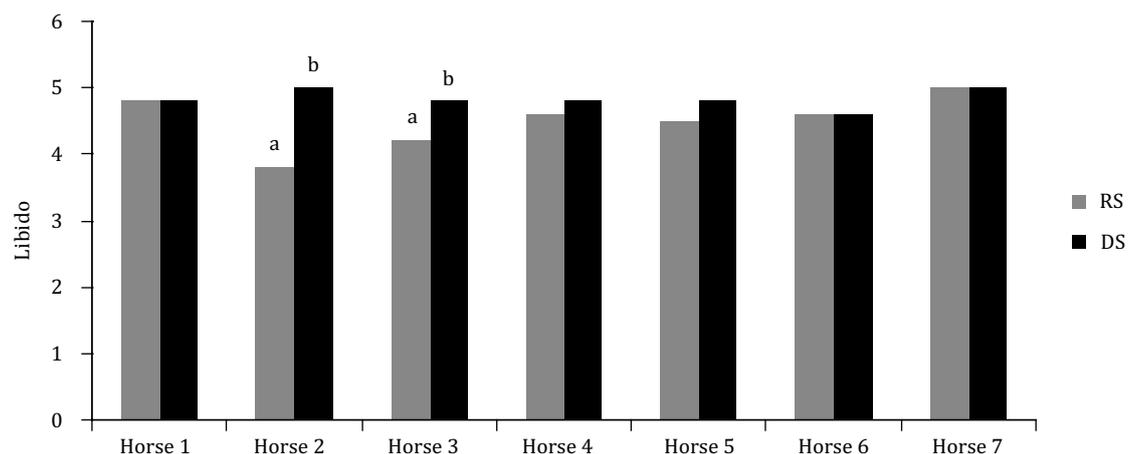
N - number of observations; % - percentage of observations.

dismount the mares. This might have occurred because the stallions are used to being allowed to run free with the mares, thereby, identifying the estrous mares and mounting them naturally without the pressure of human interference for breeding management. Then, during the experiment, the stallions were subjected to interference of a handler to lead them to the estrous mares. Furthermore, this could be the cause of the long MEjT.

The mean values of the sexual behavioral events such as MWE, MWEj, flehmen, smelling, biting, kicking, and vocalizing were low (0 ± 0.44 to 1 ± 1.39). Even with a long time to perform mounting and ejaculation, they did not court the mares with many sexual behavior events.

The most performed events were smelling and vocalizing, suggesting that they smell the mares to try to identify signals of estrus (Jeziarski et al., 2018). According to Wespi et al. (2014), the exposure of the mare to a stallion's vocalization can provide hormonal stimulation to the mare. So, the stallions' vocalizations observed in this study could be a way to stimulate signals of estrus from the females.

Despite the fact the Pantaneiro stallions took a long time to react to a mare in estrus and then to mount and ejaculate, they usually performed one mount with ejaculation, without other attempts. Lower RT and MEjT are valuable in reproductive management, although the Pantaneiro stallions in this study had the advantage of fewer attempted mounts to ejaculate, which could avoid injuries during breeding efforts.



a,b - Different letters were significantly different ($P < 0.05$) by the Kruskal-Wallis test.

Figure 1 - Libido of Pantaneiro stallions during the rainy season (RS) and dry season (DS).

Table 4 - Correlations of the sexual behaviors of Pantaneiro stallions

| Variable | Libido | RT | MEjT | MWE | MWEj | Flehmen | Smelling | Biting | Kicking |
|------------|---------|--------|--------|--------|-------|---------|----------|--------|---------|
| Libido | - | | | | | | | | |
| RT | -0.57** | - | | | | | | | |
| MEjT | -0.51** | 0.80** | - | | | | | | |
| MWE | -0.70** | 0.80** | 0.79** | - | | | | | |
| MWEj | -0.50** | 0.58** | 0.53** | 0.54** | - | | | | |
| Flehmen | -0.35* | 0.58** | 0.56** | 0.54** | 0.31* | - | | | |
| Smelling | -0.41* | 0.42* | 0.43* | 0.47** | 0.30* | 0.72** | - | | |
| Biting | -0.005 | -0.18 | -0.27* | -0.05 | 0.17 | -0.24* | -0.27* | - | |
| Kicking | -0.03 | -0.19 | -0.29* | -0.06 | 0.002 | -0.27* | -0.21 | 0.83** | - |
| Vocalizing | -0.46** | 0.16 | 0.06 | 0.22 | 0.24* | 0.26* | 0.42* | 0.26* | 0.35* |

RT - reaction time; MEjT- mounting with ejaculation time; MWE - mounting without erection; MWEj- mounting without ejaculation.

* Statistical significance ($P < 0.05$); ** highly significant ($P < 0.01$).

The stallions of this study did not present high frequencies of biting and kicking (Table 1), so they had an adequate approach to the mares. This is an advantage, considering the danger of injuries to the stallions and mares during managed breeding. In addition, this makes it less dangerous for the handler.

There was a general lack of difference in libido between the seasons, and all stallions showed good libido, classified as 4 to 5, better than the libido scores reported for stallions of other breeds (Melo et al., 1998; Rua et al., 2015, 2016; Sereno, 2002).

Two stallions showed different libido scores between the seasons. We can suggest that those animals suffered from the environmental conditions. Rua et al. (2016) reported that thermal stress with higher temperatures during breeding season (summer) can impair the libido of stallions, with longer time to mount and ejaculate, compared with the period with cooler temperatures in winter. The same might have occurred with the two Pantaneiro stallions.

The Pantaneiro stallions of this study showed longer time to mount and ejaculate comparing to the observation of Rua et al. (2015), who reported a mean of 62.7 s for Mangalarga Marchador stallions, another native Brazilian breed. However, we observed that after one week of observations, the interest of stallions in the mares decreased with a consequent rise of the time to perform mounting and ejaculation.

Even with no difference in libido between the two seasons, we observed that during the rainy season (summer), the temperature and humidity were very high, making it difficult to dissipate heat, so the stallions had intense sweating. Thus, the environment did not affect the libido of stallions but did cause some physiological responses like intense sweating, suggesting thermal stress (Sánchez et al., 2016).

One factor observed about the libido of stallions was the preference for specific mares, so we presented three mares per stallion. Two older stallions (12 and 8 years of age) usually mated with mares in the field. They only performed mounting on mares that showed strong estrus characteristics, possibly to avoid reactions like kicking and biting from mares not in estrus.

We observed that, sometimes, the stallions did not mount the mares even when they were in estrus. This finding was also reported by Isles et al. (2001) with mice and Keverne (2004) with humans, who observed that males have specific mating preferences that involve chemosensory recognition and gene expression. This selective preference occurs in many mammalian species and causes them to avoid mating with their relatives. In addition, males show attraction to the urine odors from unrelated females.

The two stallions (of eight and five years of age, respectively) that showed difference in libido between seasons (Figure 1) had better libido in the second phase of the experiment (dry season). McKinnon et al. (2011) reported that in summer (rainy season), there is a longer photoperiod phase compared with winter (dry season), causing a higher level of serum testosterone and, consequently, stronger libido. However, in this study, we observed the opposite regarding the libido. Even with the environmental changes between seasons, the stallions were able to mount and ejaculate with similar libido.

According to Curry et al. (2007), the rank within the social hierarchy may also influence reproductive success. Thus, the difference observed in these individuals could be due to the social hierarchy, in which, in the reproductive season, it presented greater competition and greater effect.

The good libido independent of season might have occurred due to the year-round availability of pastures, in turn because of the absence of a period of intense dry weather (Fernandes et al., 2010; McManus et al., 2013). Therefore, even with high mean temperatures throughout the year, the rain could avoid thermal stress, which decreases libido.

The high and negative correlations between libido and other behavioral traits (Table 4) indicates that the stronger the libido presented by the stallions, the less time they took to react to the estrous mares, the faster they mounted and ejaculated, the fewer occurrences there were of MWE and MWEj, and the less time they spent smelling and performing flehmen.

Regarding the RT, the longer the stallions took to expose the penis, the more time they spent to mount and ejaculate. On the other hand, the longer they took to be stimulated by the mare, the faster they

reacted and ejaculated. In addition, the longer they took to expose the penis, the more they attempted MWE and MWEj, and the more events of smelling and flehmen they performed. So, they spent more time courting the mare, suggesting they were testing whether the mare was in estrus. We could not say that it was weak libido because all the stallions presented high libido (scores 4 and 5).

The MEjT was negatively correlated with biting and kicking, so the longer the Pantaneiro stallions spent courting, the less aggressive they tended to be toward the females.

We observed that MWE and MWEj had high correlation with smelling and flehmen. So, the more they performed one of these traits, the more frequent those events were. The high correlation of biting and kicking showed that these aggressive traits (Losonci et al., 2016) during courting occur together. The Pantaneiro stallions of this study were not aggressive during breeding, facilitating reproductive management.

Conclusions

Pantaneiro stallions are able to mate with mares throughout the year, which can facilitate managed breeding to raise the number of Pantaneiro horses and optimize the use of the stallions during the breeding season, with more females per male.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.A.S. Ribas, I.C.N. Cunha and C.R. Quirino. Formal analysis: A. Bartholazzi Junior and C.R. Quirino. Funding acquisition: C.R. Quirino. Investigation: M.A.S. Rua, J.A.S. Ribas, J.F.S. Silva, I.C.N. Cunha, A. Bartholazzi Junior, A.R.M. González and C.R. Quirino. Methodology: M.A.S. Rua, J.A.S. Ribas, J.F.S. Silva, I.C.N. Cunha, A. Bartholazzi Junior, A.R.M. González and C.R. Quirino. Project administration: J.A.S. Ribas and C.R. Quirino. Supervision: C.R. Quirino. Visualization: J.A.S. Ribas and C.R. Quirino. Writing-original draft: M.A.S. Rua. Writing-review & editing: J.A.S. Ribas, J.F.S. Silva, I.C.N. Cunha, A. Bartholazzi Junior, A.R.M. González and C.R. Quirino.

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